REVIEW ARTICLE

Tau pathology: predictive diagnostics, targeted preventive and personalized medicine and application of advanced research in medical practice

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Abstract Microtubules are key cytoskeletal elements found in all eukaryotic cells. The microtubule shaft is composed of the heterodimer protein, tubulin and decorated with multiple microtubule associated protein, regulating microtubule function. Tau (tubulin associated unit) or MAPT (microtubule associated protein tau), among the first microtubule associated proteins to be identified, was implicated in microtubule initiation as well as assembly, with increased expression in neurons and specific association with axonal microtubules. Alzheimer's disease (AD) is the most prevalent tauopathy, exhibiting tau-neurofibrillary tangles associated with cognitive dysfunction. AD is also characterized by β-amyloid plaques. An abundance of tau inclusions, in the absence of β-amyloid deposits, can be found in Pick's disease, progressive supranuclear palsy (PSP), corticobasal degeneration (CBD) and other diseases, collectively described as tauopathies. The increase in tau pathology in AD correlates with the associated cognitive decline. The current manuscript touches on the variability as well as common denominators of the various tau pathologies coupled to new approaches/current innovation in treatment of tauopathies in favor of advanced technologies in predictive diagnostics, targeted preventive and personalized medicine (PPPM).

Keywords PPPM · Tauopathy · Tau · MAPT · Alzheimer's disease · Frontotemporal dementia

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Tau background

The understanding of the multiple triggers of neurodegeneration is bound to lead to better diagnostics and improved therapeutics. In this respect and looking for unifying pathways, the accumulation of intracellular neurofibrillary lesions composed of abnormally phosphorylated and aggregated tau protein was identified as characteristic in many neurodegenerative disorders [1].

The microheterogeneity of tubulin [2, 3], the building block of the microtubule shaft, expressing multiple isotypes with brain specificity [4], coupled to the intricate complexity of the microtubule interacting proteins leads to enhanced control of neuronal function. Mis-metabolism and mutations in tubulin or in the interacting proteins leads to a variety of disease conditions, e.g. [5]. Here the emphasis will be put on the tubulin interacting protein, tau.

Tau (tubulin associated unit) or MAPT (microtubule associated protein tau) [6] is required for microtubule initiation as well as assembly [7], with increased expression in neurons and specific association with axonal microtubules [8]. MAPs binding to tubulin/microtubules enables them to play a fundamental role in promoting microtubule assembly and stability [6, 9, 10]. Functionally, microtubules are essential for cell division, neuronal development, maintenance of neuronal shape, neuronal plasticity (e.g. plasticity of dendritic spines and axoplasmic transport with tau participating in neurite extension and axonal transport [11–14].

Tau is primarily a neuronal protein, though not exclusively. In an adult human brain, there are six major isoforms of tau derived from a single gene through alternative splicing [15, 16]. These isoforms differ by the presence or absence of one or two short inserts in the amino terminal half and in whether they contain three or four tubulin binding domain repeats in the carboxy terminal

half. The alternative splicing of tau has been suggested to impact axonal functional homeostasis [17], and 3 repeat tau was suggested to have a stronger effect on axon transport dynamics.

Using cultured Aplysia neurons and online confocal imaging of human tau, it was shown that over-expression of tau generates the hallmarks of human tau pathogenesis. It was further demonstrated that the tau-induced impairment of organelle transport is because of polar reorientation of the microtubules along the axon or their displacement to submembrane domains establishing that tau over-expression leads to impaired retrograde and anterograde organelle transport [18].

The following review is an update on a review in press in Current Alzheimer Research [19] it is not a comprehensive review of the literature, but rather a point of view touching upon some recent publications as follows below.

Tauopathies

Disruption of the microtubule network is a hallmark of neurodegeneration, including the most prevalent tauopathy, Alzheimer's disease (AD). AD is characterized by the deposition of intracellular fibrillar structures forming paired helical filaments (PHFs) followed by larger aggregates termed neurofibrillary tangles (NFTs) [20] as well as the β-amyloid deposits.

Tau was originally identified as the primary component of PHF [21–27]. NFTs are composed mainly of PHFs with a minority of straight filament (SF), [28–30]. PHFs appear to consist of two filaments that are wound helically around one another, with a longitudinal spacing between cross-overs of about 80 nm and a width of 30 nm at the widest point and 15 nm at the narrowest [31]. PHFs morphology distinguishes them from microtubules (250 nm diameter), neurofilaments (~100 nm diameter) and microfilaments (~60 nm diameter) which are the major cytoskeletal elements in healthy neurons [28–30, 32]. The biochemistry of tau is a subject of multiple reviews [33].

Phosphorylation occurs on a number of different parts of tau and an increase in phosphorylation generally reduces tau-tubulin binding. A link to a slide delineating different tau domains and tau mutations can be accessed via: http://www.alzforum.org/res/com/mut/tau/table1.asp. This site outlines the various mutations that have been found in tau that are associated with multiple tauopathies including CBD = corticobasal degeneration. DDPAC = disinhibition-dementia-parkinsonism-amyotrophy complex; FTD = frontotemporal dementia. FTDP-17 = FTD with Parkinsonism linked to chromosome 17; HFDT = hereditary frontotemporal dementia; MSTD = multiple

system tauopathy dementia; PPND = pallidopontonigral dementia; and PSP = progressive supranuclear palsy. The different tau mutations are associated with different morphology of the tau pathology (http://www.alzforum.org/res/com/mut/tau/table1.asp). The original identification of mutations in tau in familial forms of FTD was described in 1998 [34–37].

The majority of tau in PHFs and NFTs is hyper-phosphorylated [38, 39]. This hyperphosphorylation maybe related to either an increase in kinase activity or a decrease in phosphatase activity [40]. Additionally, tau undergoes a specific type of serine—threonine O-glycosylation, and these modifications can reduce the extent of tau phosphorylation [41]. Tau can also be tyrosine phosphorylated [42], sumoylated and nitrated [43], although the effects of these modifications on tau require further investigations. Tau hyperphosphorylation appears to precede its accumulation in the affected neurons in AD [44, 45]. Hyperphosphorylated tau shows impaired axonal transport [12, 46], defective microtubule binding [47–50], failure to promote microtubule assembly [51, 52], and self-assembly into NFTs [53, 54]. Additionally, hyperphosphorylation of tau might make it more resistant to caspase proteolysis as well as degradation and thereby more likely to accumulate in neurons, forming PHFs and NFTs [55].

As suggested above, original studies indicated that a down-regulation of protein phosphatase 2A (PP2A), the major tau phosphatase in human brain, contributes to tau hyperphosphorylation in AD. Importantly, PP2A dephosphorylated tau at several phosphorylation sites with different efficiencies. Among the sites studied, Thr205, Thr212, Ser214, and Ser262 were the most favorable sites, and Ser199 and Ser404 were the least favorable sites for PP2A in vitro. In addition to its direct effect on tau, inhibition of PP2A with okadaic acid in metabolically active rat brain slices caused inhibition of glycogen synthase kinase-3beta (GSK-3beta) via an increase in its phosphorylation at Ser9. GSK-3beta phosphorylates tau at many sites, with Ser199, Thr205, and Ser396 being the most favorable sites. The overall alterations in tau phosphorylation induced by PP2A inhibition were the result of the combined effects of both reduced tau dephosphorylation due to PP2A inhibition directly and reduced phosphorylation by GSK-3beta due to its inhibition [56]. Tau phosphorylation impacts its biological activity and neurofibrillary degeneration in association with the specific phosphorylation site (for a comprehensive web site on tau hyperphosphorylation, including the kinases involved see (http://www.alzheimer-adna.com/Gb/Tau/TauPhosphoSeq.htm). Thus, the interplay between phosphorylating enzymes and phosphatases plays a role in tauopathy progression.

Most of the tau phosphorylation sites that have been characterized were Ser and Thr residues. More recent reports showed that tau can be phosphorylated at Tyr residues by kinases including Fyn, Syk, and c-abl (Abl). Proteomic analyses show that tau phosphorylated at Tyr394 (Y394) exists within AD PHF samples. It was further shown that Abl phosphorylated this particular site on tau. A most recent report showed that Arg, the other member of the Abl family of tyrosine kinases, also phosphorylates tau at Y394 in a manner independent of Abl activity. Given the reported role of Arg in oxidative stress response and neural development, the ability to phosphorylate tau at Y394 implicated Arg as a potential player in the pathogenesis of AD and other tauopathies [57].

The MAPT gene on human chromosome 17 appears in two main extended haplotypes. In a total of approximately 200 unrelated Caucasian individuals, there was complete disequilibrium between polymorphisms which span the gene (which covers approximately 100 kb of DNA). This showed an establishment of the two haplotypes (H1 and H2) [58]. It was further shown that the more common haplotype (H1) is significantly over-represented in PSP patients [59].

Single nucleotide polymorphisms mapped linkage disequilibrium in the regions flanking MAPT and have established the maximum extent (~2 Mb) of the haplotype block on chromosome 17 q21.31 [60] and a 900 kb inversion which suppresses recombination [61]. This ~2 Mb gene-rich region extends centromerically slightly beyond (~400 kb) the corticotrophin releasing hormone receptor 1 gene. The telomeric end was defined by a ~150 kb region beyond the WNT3 gene. This study of Pittman et al., showed that the entire H1 haplotype is associated with PSP, which may implicate several other genes in addition to MAPT, as candidate pathogenic loci [60]. Additional studies by Pittman et al., resolved multiple variants of the H1 haplotype, reflecting a greater diversity of MAPT than can be explained by the H1 and H2 clades alone [62].

Mild cognitive impairment (MCI) is often considered a transitional condition prodromal to AD. Therefore, the genotypes of 7 polymorphisms tagging the major tau haplotypes were assayed on 186 patients with amnestic MCI and 191 unrelated controls. Association study was conducted by logistic regression including apoliprotein E genotype and age as covariates (with the aplipoprotein E4 and age representing major risk factors for AD). The common H1 haplotype was found to be significantly overrepresented in amnestic MCI patients. This finding was confirmed when the aplipoprotein E4 allele was taken into account. These results suggest 1] that the risk of MCI is influenced by tau protein gene variations and 2] that MCI

shares a common genetic background with AD. The results may help elucidating the genetic risk to cognitive decline and designing effective clinical trials, future diagnostics and future therapies [63].

Interestingly, in trying to evaluate the rate of progression and the predictors of worsening in frontotemporal lobar degeneration (FTLD) patients, 127 FTLD patients entered a study and were re-evaluated at 1-year follow-up. A statistical driven approach on wide neuropsychological, behavioral, and functional data was applied to identify homogeneous groups both at baseline and at follow-up within FTLD, taking into account: (i) the demographic characteristics, (ii) the genetic background, i.e. apolipoprotein E genotype, tau haplotype, and functional polymorphisms affecting serotonin and dopamine pathways, and (iii) the clinical phenotype. On the basis of the overall assessment (disease severity), the results recognized two groups of patients, "good performers" and "bad performers". At 1-year follow-up, almost 30% of FTLD patients progressed from "good" to "bad" performances, whilst 70% maintained stable "good" performances. Apolipoprotein E4 allele, Tau H2 haplotype and behavioral variant FTD phenotype were associated with worse prognosis over time, suggesting specific genetic and clinical predictors in FTLD progression [64] that converge and differ from those of other tauopathies (e.g. amnestic MCI/AD and PSP).

Recent reviews describe the different tauopathies, e.g. [33, 65]. Thus, tau comprises the Pick bodies found in Pick's disease (PiD) with aggregates of three microtubule binding repeat tau (3R tau) and frontal atrophy associated with cognitive clinical dysfunction of frontal dysexcutive syndrome, progressive nonfluent aphasia and semantic dementia [33, 65].

Like other tauopathies, progressive supranuclear palsy (PSP) is characterized by accumulation of abnormally phosphorylated tau [66, 67]. Predominantly, the four microtubule binding domain (4R) form of tau accumulates in straight NFTs in neurons and glial cells in affected brain regions [59, 67, 68]. PSP is characterized by NFTs in basal ganglia, diencephalon, and brain stem with frontal executive cognitive impairment and movement disorders characterized by supranuclear gaze palsy, falls and Parkinsonism-like behavior [65, 69–71]. It is a rare progressive disease [72–74]. Like in AD and PiD, brain atrophy occurs in patients with PSP. While some degree of brain atrophy occurs with normal aging, the atrophy in PSP is more pronounced [75, 76], correlating with clinical disease progression [77] and assessed by structural brain imaging [75, 78].

Cortico-basal ganglionic degeneration (CBD) is characterized by parietofrontal or frontotemporal atrophy and pallor in substantia nigra and 4R tau-aggregates (also found in glial lesions) that is manifested by cognitive dysfunction —

cortical sensory loss, apraxia (loss of the ability to execute or carry out learned purposeful movements) and asymmetric akinetic rigid syndrome [33, 65].

PiD, PSP and CBD are subtypes within the broader category of called frontotemporal dementias (FTD). Another FTD is frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17) that is characterized by frontal atrophy often seen with tau-positive neuronal and glial inclusions with behavioral changes, cognitive decline and Parkinsonism [34].

Many of the tau alterations that are characteristic of AD have also been identified in PSP and CBD. Tau truncation at Asp421 is an alteration that is unique to neuronal lesions, occurring in Pick bodies as well as in NFTs, but not in lesions associated with glia. Conversely, phosphorylation at Ser422 is not only present in all these lesions, but identifies additional glial and neuronal pathology in disease-susceptible cortical regions. The results suggest that the molecular alterations of tau that occur during the initial process of tangle formation in AD are similar in non-AD tauopathies, but the middle and later changes are not common to all diseases [79].

Tauopathies include also the early-onset dementia observed in Down syndrome (DS; trisomy 21). Splicing misregulation of adult-specific exon 10 results in expression of abnormal ratios of tau isoforms, leading to FTDP. Positions +3 to +19 of the intron downstream of exon 10 define a hotspot: Point mutations in it result in tauopathies. All these mutations increase the inclusion of exon 10 except for mutation +19, which almost entirely excludes exon 10. To investigate the tau connection between DS and AD, a recent study examined splicing factors located on chromosome 21 for their effect on tau exon 10. In these experiments, the splicing factor located on chromosome 21, heterogeneous nuclear ribonucleoprotein E3 — hnRNPE3 (PCBP3), was found to modestly activate the splicing of exon 10 by interacting with its proximal downstream intron around position +19 [80]. Other studies found decreased levels of Tra2beta, an RNA splicing factor responsible for tau exon 10 inclusion, in both cortical cell cultures exposed to MG132 (proteasome inhibitor) and in cerebral cortex after ischemic injury, suggesting that transient focal cerebral ischemia reduces tau exon 10 splicing through a mechanism involving proteasome-ubiquitin dysfunction and down-regulation of Tra2beta [81].

Diagnostic tools

Cerebrospinal fluid (CSF) total tau levels vary widely in neurodegenerative disorders, thus not being useful in their discrimination over AD. It has been suggested that total tau

alongside with amyloid beta can predict conversion from MCI to AD [82–85]. AD is characterized by a signature of phosphorylated tau and amyloid beta in the CSF [84], coupled with brain imaging technologies this can follow AD disease progression [86–88] (http://www.labtestsonline.org/understanding/analytes/tau/test.html). Commercial companies like Applied NeuroSolutions, Inc. (http://www.appliedneurosolutions.com/) and Innogenetics (http://www.innogenetics.com/neurodegeneration.html) offer measurements of phosphorylated tau in the CSF, specifically P-tau 231 and P-tau 181 determination, respectively. Furthermore, a novel strategy to characterize tau versions present in CSF with respect to their molecular mass and isoelectric point was just published which will facilitate advanced diagnosis [89].

A recent study characterized and measured tau forms in order to verify the differential patterns among neurodegenerative disorders. A quantitative immunoprecipitation was developed showing an extended (55 kDa), and a truncated (33 kDa) forms of tau in the CSF with differential expression. Thus the tau ratio 33 kDa/55 kDa was significantly decreased in patients with PSP (0.46+/−0.16) when compared to controls, including healthy subjects (1.16+/−0.46, $P=0.002$) and AD (1.38+/−0.68, $P<0.001$), and when compared to FTD (0.98+/−0.30, $P=0.008$) or CBD (0.98+/−0.48, $P=0.02$). Moreover, in PSP patients tau form ratio was lower than in other neurodegenerative extrapyramidal disorders, such as Parkinson disease (1.16+/−0.26, $P=0.002$) and dementia with Lewy bodies (1.44+/−0.48, $P<0.001$). Tau ratio 33 kDa/55 kDa did not correlate either with demographic characteristics, cognitive performances or with motor impairment severity. Truncated Tau production shows a different pattern in PSP compared to other neurodegenerative disorders, supporting the view of disease-specific pathological pathways. These findings are promising in suggesting the identification of a marker for PSP diagnosis in clinical practice [90].

Another study suggested that CSF concentrations of the 42 amino acid fragment of amyloid-beta (Abeta42), neurofilament light chain (NFL), neurofilament heavy chain (pNFH), tau protein, glial fibrillary acidic protein (GFAP), neuron specific enolase (NSE), S-100B protein, and myelin basic protein (MBP) that are released into the CSF after brain tissue damage caused by a variety of neurological diseases could be of value in the differential diagnosis of neurodegenerative disorders [91].

In an elegant study 18 signaling proteins in blood plasma were suggested to be used to classify blinded samples from AD and control subjects with close to 90% accuracy and to identify patients who had MCI that progressed to AD 2–6 years later. Biological analysis of the 18 proteins points to systemic dysregulation of hematopoiesis, immune

responses, apoptosis and neuronal support in presymptomatic AD. Further studies should evaluate whether this test could be implemented for other tauopathies and whether distinction can be made based on this highly desirable plasma evaluation tool [92].

A recent study also used a molecular imaging probe for plaques and tangles, 2-(1-{6-[(2-[F-18]fluoroethyl)(methyl) amino]-2-naphthyl}ethylidene)malonon itrile ([(18)F] FDDNP) relating cognitive ability to positron emission tomography (PET) and suggesting this an a potential early diagnostic tool [93].

Tau as a drug target is a relatively young field and future clinical studies should aim at evaluating tau distribution in biological fluids as well as develop imaging technologies to identify potential changes in tau following candidate drug application.

Activity-dependent neuroprotective protein (ADNP), tauopathy and neuroprotection

Our own focused research is on activity-dependent neuroprtective protein (ADNP). When we discovered ADNP [94, 95] and as I have recently reviewed [96], bioinformatics suggested that ADNP is a transcription factor containing a homeobox domain profile with sequence motifs that are associated with nuclear localization as well as cellular secretion and uptake. These structural characteristics imply nuclear, cytoplasmic and extracellular functions [94, 95]. When we performed complete knockout of ADNP in the mouse, our results revealed cranial neural tube closure failure and death on E8.5–9.0 of the ADNP-knockout embryos [97]. To further elucidate ADNP associated pathways, we used Affymetrix microarrays on ADNP knockout and control mouse embryos (E9), resulting in gene expression changes of >450 genes. A group of dramatically up-regulated gene transcripts in the ADNP-deficient embryos were clustered into a family encoding for proteins enriched in the visceral endoderm such as apolipoproteins (including apolipoprotein E), cathepsins and methallotionins. A down regulated gene cluster associated with ADNP-deficiency in the developing embryo consisted of organogenesis markers including neurogenesis (Ngfr, neurogenin1, neurod1) and heart development (Myl2) [98]. Our results placed ADNP at a crucial point of gene regulation, repressing potential endoderm genes and enhancing genes associated with organogenesis/neurogenesis. Immunoprecipitation experiments showed interactions with heterochromatin protein1α (HP1α) [98] and with BRG1, BAF250a, and BAF170, all components of the SWI/SNF (mating type switching/sucrose nonfermenting) chromatin remodeling complex [99]. Together, our results place ADNP in a chromatin remodeling epigenetic role in neurodifferentiation and neuroplasticity and aging.

While complete ADNP-deficiency is lethal, we have shown that ADNP heterozygous mice (+/−) survive, but exhibit phenotypic deficiencies. ADNP+/− male mice exhibited cognitive deficits, significant increases in phosphorylated tau, tangle-like structures emanating from astrocytes (as described for CBD and PSP) and neurodegeneration as compared to ADNP+/+ mice [100]. It is an open question as to whether ADNP is directly associated with human tauopathies.

Interestingly, comparison of the expression of ADNP mRNA in the peripheral blood mononuclear cells (PBMCs, i.e. T-cells, B-cells, monocytes and natural killer cells) of normal subjects and multiple sclerosis patients showed that monocytes, B-cells and T-cells, but not regulatory (CD4 + CD25+) T-cells expressed ADNP that was reduced in the PBMCs of multiple sclerosis patients compared to those of the healthy controls. The authors suggested that the decreased expression of ADNP in PBMCs of multiple sclerosis may contribute to reduced immuno-regulatory capacity in these patients [101]. Other studies showed increased tau hyperphosphorylation in multiple sclerosis [102].

## Drug candidates aimed at tauopathy [19, 103–105]

Several aspects of tauopathy are currently targeted. Those include, targeting tangles to break the potentially toxic aggregates, inhibiting tau phosphorylation, accelerating tau dephosphorylation, and accelerating — tau microtubule interactions (for review please see [106]).

Advanced models of tauopathy have been developed for translational research, including a model for rapid analysis of tau-related degeneration in zebrafish [107], a model of tauopathy in drosophila that dissociates tau toxicity and phosphorylation at the level of GSK-3beta, MARK and Cdk5. The model suggests that, in addition to tau phosphorylation, microtubule binding plays a crucial role in the regulation of tau toxicity when misexpressed. These data have important implications for the understanding and interpretation of animal models of tauopathy [108]. Advanced mouse models are many as recently reviewed [109]. Preferred models include models where the transgene can be inactivated at will (e.g. [110]). Interestingly, the propagation of protein misfolding, such as tau, may occur through mechanisms similar to those that underlie prion pathogenesis [111–113].

Non-related studies have shown tau enrichment in metastatic tumors and an ability of tau to promote tumor cell reattachment through tubulin microtentacles formation,

supporting a model in which tau-induced microtubule stabilization provides a selective advantage during tumor metastasis [114] and suggesting a potential cross talk between cancer therapeutics/diagnosis and tauopathies.

Our research demonstrated that an 8-amino-acid peptide fragment of ADNP was capable of conferring neuroprotection. This peptide fragment is called davunetide (also known as NAP) and has the following amino acid sequence:

$$H_2N - Asn - Ala - Pro - Val - Ser - Ile - Pro - Gln - COOH$$
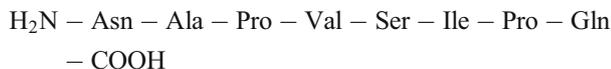
Preclinical experiments indicate that davunetide has neuroprotective, cognitive protective, and neurotrophic properties. Importantly, NAP (davunetide), did not affect dividing cells (unlike paclitaxel and other microtubule targeting drugs) [115].

We have indentified NAP (davunetide) as a potent neuroprotectant in a wide range of in vitro models [116] against a number of toxic insults including several relevant to neurodegenerative diseases such as amyloid beta peptide [94, 117], excitotoxicity [94], oxidative stress [118] and oxygen glucose deprivation associated apoptosis [119]. We have further identified NAP (davunetide) as a neurotrophic factor, stimulating neurite outgrowth and synapse formation [120]. These results were corroborated by other investigators worldwide [121–123].

To understand the biological significance of NAP (davunetide) activity, we have generated mice partially deficient of the NAP (davunetide) -containing protein, ADNP [100]. As indicated above, while the complete knockout embryos do not form a brain and die in utero [97, 99], the heterozygous mice live and exhibit severe learning deficiencies which are ameliorated, in part, by intranasal NAP (davunetide) treatment [100]. Tau hyperphosphorylation occurs in these ADNP deficient mice and is reduced by NAP (davunetide) treatment [100]. These studies demonstrate the functionally significant role of ADNP and NAP (davunetide) in limiting tau hyperphosphorylation.

As indicated above, multiple sclerosis may also be associated with tauopathy [102] and recent studies associated reduced ADNP in the altered immune capacity of the patients which maybe compensated by NAP (davunetide) treatment [101, 124].

We have further shown activity for NAP (davunetide) in a number of transgenic mouse models of dementia including AD and tau mutations. In one of our most recent studies, chronic intranasal NAP (davunetide) treatment has been shown to reduce neurofibrillary tangles and tau hyperphosphorylation in a "pure" tauopathy model that has direct relevance of FTD [125]. This double transgenic mouse model developed by our collaborator Dr. Rosenmann at Hadassah Hospital in Israel has two mutant tau transgenes

(P301S; K257T) under the control of the tau promoter [126]. The mice develop inclusions in the hippocampus and cortex accompanied by cognitive and behavioral dysfunction. These mutations (reviewed in [127]) are both known to cause familial forms of FTD with variable features of PSP, CBD and less frequently amyotrophy, due to the tau proteins having a reduced ability to stabilize microtubules. We treated the tau transgenic mice with intranasal NAP (davunetide) over several months which resulted in reduced tau phosphorylation and tangle pathology paralleled with improved short-term spatial learning and memory [125]. Our results indicate that long-term NAP (davunetide) treatment associated with reduction in tau pathology may improve cognitive function and slow disease progression.

The triple transgenic mouse model of AD expressing mutant APP (Swedish), tau (P301L), and presenilin-1 (M146V) develops both neurofibrillary tangles and amyloid beta plaques in a progressive fashion [128]. When we treated 12-month-old animals with an intranasal dose of 2 μg/day (~0.07 mg/kg/day) for 3 months our results showed a 70% decrease in phosphorylated tau at Ser202/Thr205, Thr231, and Ser202 residues [129, 130]. Histological examination of the hippocampal CA1 region confirmed that NAP (davunetide) treatment resulted in a reduction of phosphorylated tau. Treatment of 9-month-old animals with an intranasal dose of 0.5 μg/day (~0.017 mg/kg/day) for 3 months resulted in a 30% to 40% decrease in phosphorylated tau.

In humans, davunetide intranasal (AL-108) has been studied for the treatment of amnestic MCI. The study was a randomized, double-blind, placebo-controlled, parallel group study. The effect of davunetide 5 mg once daily and 15 mg twice daily compared to placebo was evaluated in several tests of cognitive function. In that study, 144 subjects were randomized and 125 subjects completed the study. Subjects treated with davunetide 15 mg twice daily demonstrated a general pattern of improvement in cognitive tests that primarily assessed attention and working memory function. Both doses of davunetide were safe and well tolerated. Headache and nasopharyngeal symptoms were the most commonly reported adverse events [131]. These studies place tau and tauopathies at central stage for further understanding of neurodegenerative diseases and the development of neuroprotective drugs.

Following our discovery of NAP (davunetide, [94]) we have suggested that NAP (davunetide) affects microtubule stability thereby providing neuroprotection [132, 133]. Our recent studies extended the breadth of NAP (davunetide) application to show that it protected against cognitive dysfunction in a schizophrenia model of microtubule deficiency [134]. Importantly, clinical studies in schizophrenia patients suffering from cognitive impairment performed by TURNS (Treatment Units for Research on

Neurocognition and Schizophrenia) in collaboration with Allon Therapeutics Inc. (www.allontherapeutics.com) showed protection of functional capacity (activities of daily living) and protection of brain function (magnetic resonance spectroscopy measurements of N-acetyl aspartate) in the treated patients (Javitt et al., in preparation).

Based on the animal translational studies and the human clinical efficacy, davunetide is now poised for further clinical studies in PSP, an orphan indication (Allon Therapeutics, Inc.).

Paclitaxel and related compounds have been suggested to act also in protecting microtubules, however, bioavailability, brain specificity and the potential irreversibility of their action suggests that more work is required prior to paclitaxel-like neuroprotective drug candidate [135]. A drug candidate in a similar stage of development to davunetide is rember (methylene blue) that was featured in several AD meetings as per [104, 136].

In the preclinical stage, a phenylthiazolyl-hydrazide (PTH) compound was suggested as a possible hit in terms of inhibition of tau aggregation and the core of the PTHs crucial for activity was identified, thus representing a putative lead structure [137]. Recently, quantitative high-throughput screening (qHTS) of approximately 292000 compounds to identify drug-like inhibitors of tau assembly was developed. The fibrillization of a truncated tau fragment that contains four MT-binding domains was monitored in an assay that employed complementary thioflavin T fluorescence and fluorescence polarization methods. Previously described classes of inhibitors as well as new scaffolds were identified, including novel aminothienopyridazines (ATPZs). A number of ATPZ analogues were synthesized, and structure-activity relationships were defined. Further characterization of representative ATPZ compounds showed they do not interfere with tau-mediated MT assembly, and they are significantly more effective at preventing the fibrillization of tau than the Abeta(1–42) peptide which forms AD senile plaques. Thus, the ATPZ

molecules are suggested for further development [138]. As a follow-up on these publications, AstraZeneca and The University of Pennsylvania recently announced a new collaborative research agreement that initially will focus on generating new AD drug candidates for the clinical development pipeline (http://www.astrazeneca.com/research/?itemId=8876304).

Various tau aggregation inhibitory molecules were recently reviewed [104] also taking into consideration that the cellular environment affects tau aggregation. In this respect, it has been suggested that tau fragments across the lysosomal membrane promote formation of tau oligomers at the surface of these organelles which may act as precursors of aggregation and interfere with lysosomal functioning [139].

Other aspects of tau-related future therapeutics involve targeting tau hyperphosphorylation [140] with inhibitors of the key tau phosphorylating enzymes such as Cdk5/p25 kinase [141], GSK-3 inhibitors, such as lithium [142] or peptide inhibitors [143] and possible enhancement of protein phosphatase-2A (PP-2A) [144]. Other kinases implicated in tau phosphorylation include CK1, PK1, MARK, and the stress associated kinases, p38MAPK and JNK [140]. ATP competitive inhibitors may present potent drug candidates, but may also have undesirable side effects [106].

Regarding lithium, it is currently in clinical trials in PSP and CBD (http://clinicaltrials.gov/ct2/show/NCT00703677?term=lithium&rank=6) and was tested in Alzheimer's disease patients (http://clinicaltrials.gov/ct2/show/NCT00088387?term=lithium+alzheimer%27s+disease&rank=1).

NP12 (Nypta®), a GSK-3 inhibitor [145], that we have reviewed in our paper "Looking for novel ways to treat the hallmarks of Alzheimer's disease" as microtubule — related drug candidate [105] is a potent thiadiazolidinone derivative, that when injected into the rat hippocampus dramatically reduces kainic acid-induced inflammation, as measured by edema formation using T_2-weighted magnetic

Table 1 Drug candidates

Drug candidate	Activity
Davunetide (NAP)	Microtubule protection: preventing tau hyperphosphorylation and tau aggregate formation and providing neuro-glial protection
Aminothienopyridazines (ATPZs)	Preventing fibrillization of tau
Rember (methylene blue) and derivatives	Preventing tau aggregation
GSK-3 inhibitors:	
• lithium	
• NP-12 (Nypta®)	Preventing tau phosphorylation
Rasagiline mesylate	Neuroprotection
Immunotherapy	Anti-tau antibodies Potential personalized medicine — future potential for targeting of specific mutations

Other drug candidates of similar (or different) categories as well as innovative approaches are described in the text

resonance imaging and glial activation and has a neuro-protective effect in the damaged areas of the hippocampus [146].

NP-12 (Nypta®), is claimed to be the first non ATP competitive inhibitor of GSK3, has been already administered to >140 healthy volunteers to date, as presented at the International Conference on Alzheimer's Disease (ICAD) in 2008. NP-12 is developed by Noscira (formerly Neuropharma, S.A.). Updates are available on the company website: "The first Phase II trial for Alzheimer's was approved in the last quarter of 2008; 30 patients have already been treated, and the results are being processed. Noscira expects the first Phase II clinical trial in PSP to begin in 2009."

Regarding kinase inhibition, internet search of SAR-502250 revealed the patent application: WO/2009/035159 also including MITSUBISHI TANABE PHARMA CORPORATION in the applicants. The patent abstract describes a compound represented by a formula that is disclosed or a pharmaceutically acceptable salt thereof: which is used for preventive and/or therapeutic treatment of a disease caused by abnormal activity of tau protein kinase 1 such as a neurodegenerative diseases (e.g. Alzheimer disease) (http://www.wipo.int/pctdb/en/wo.jsp?WO=2009035159). Furthermore, SRN-003-556 was described as an inhibitor of tau hyperphosphorylation that prevents severe motor impairments in tau transgenic mice [147].

Brunden et al., also review the possibility of increased tau degradation as a therapeutic target also looking at hsp90 [148] as well as macroautophagy [139]. Neuroprotection is another approach to another disease associated with tau deregulation, A clinical trial with patients with Multiple System Atrophy (MSA), a disease with tau inclusions [149] and with parkinsonism is currently ongoing (http://clinical trials.gov/ct2/show/NCT00977665).

Another approach to clearing tau aggregates has been tau immunotherapy [150, 151]; this approach is still in the preclinical stage, showing potential promise in animal studies. Yet, another approach to the regulation of tau could be specific inhibition of synthesis, when this seems to lead to accumulation as well as addressing specific tau mutations (please see above and as described in http://www.alzforum.org/res/com/mut/tau/table1.asp). When RNA silencing and targeted in vivo mutagenesis further develop to potential candidate drugs, this would be an interesting avenue to follow. Finally, given the preponderance of 4 repeat (4R) tubulin binding domain tau in the neurodegenerative brain, drugs that target enhancement in the expression of the 3R tau to restore homeostasis are highly desirable, however, keeping in mind that the homeostasis between the 3R and 4R tau isoforms is important and that 3R tau has been associated with the pathology of Pick's disease [33, 65].

Additional reading on the subject can be found is several reviews including, but not limited to the following literature citations [106, 152] and including insights to the selection of the patient populations [153], some of the drug candidates outlined above are summarized in Table 1.

Future outlook

In terms of prediction, the tau mutations, amyloid precursor protein (APP) mutations, presenilin mutations and Down's syndrome predict tauopathy. Single nucleotide polymorphism (SNP) signatures may also contribute to disease susceptibility and progression. Furthermore, the tau gene haplotype contributes to susceptibility as well as additional risk genes like apoliprotein E4. Thus, genetic profiling will address prediction and provide for future diagnosis.

A very interesting facet of these diseases is the late onset; it is also possible that the initial progress is slower until it reaches a point of no return and is accelerated, perhaps due to accumulations of stress signals. In terms of diagnosis, one way is to follow-up plasma/blood and CSF samples of family members — carriers of mutations, heterozygous and non-carriers for tau abnormalities, changes in concentration, changes in degree of phosphorylation and truncation to address disease progression and potential preventative measures.

Personalized medicine in this respect will include customized immunotherapy as well as gene therapy, shutting down the mutated gene and introducing the healthy gene, using most advanced molecular genetic tools. Personalized treatment will stem from knowledge of the specific mutation, alteration in gene expression and post-translational processing, toward a brighter future.

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